PATENT

A1

The application is a continuation-in-part of U.S. Patent Application Serial Number 09/784,866 filed on February 15, 2001 which claims priority to U.S. Provisional Patent Application Serial Number 60/182,844 filed on February 16, 2000. The application also claims priority to U.S. Provisional Patent Application Serial Number 60/211,054 filed on June 13, 2000. These disclosures are incorporated herein in their entirety for all purposes.

IN THE CLAIMS:

Please amend claims 1, 2-6, 8-9, 15-17, and 18 as follows. Please add new claims 19-31.

A2

1. (Once amended) A method of detecting the presence of a single copy of a target nucleic acid in a sample, said method comprising:

detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy of said target nucleic acid, wherein said first quantum dot and said second quantum dot are distinguishable, thereby detecting said single copy of said target nucleic acid.

A3

2. (Once amended) The method as in claim 1, further comprising quantitating the target nucleic acid by analyzing the detected optical characteristic.

3. (Once amended) The method as in claim 1, further comprising transcribing the target nucleic acid.

4. (Once amended) The method as in claim 3, wherein the target nucleic acid comprises DNA and transcribing comprises using a primer which anneals to a conserved region of the DNA and transcribes a polymorphic region of the DNA when extended.

5. (Once amended) The method as in claim 4, wherein the primer is biotinylated and the transcribing step produces biotinylated DNA.

A3

6. (Once amended) The method as in claim 3, further comprising binding the transcribed target nucleic acid to a substrate.

A4

8. (Once amended) The method as in claim 6, further comprising removing unbound portions of the target nucleic acid.

9. (Once amended) The method as in claim 6, further comprising probing the bound target nucleic acid using a sequence-tagged hybridization probe.

A5

15. (Once amended) The method as in claim 13, wherein detecting comprises scanning the substrate with resolution capable of detecting an optical characteristic of a single quantum dot.

16. (Once amended) The method as in claim 15, further comprising quantitating the target nucleic acid by analyzing the detected optical characteristic, wherein analyzing comprises counting the number of quantum dots within an area of scanned substrate.

17. (Once amended) A method of detecting the presence of a single copy of a target nucleic acid in a sample, said method comprising:

detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy of said target nucleic acid, wherein said first quantum dot and said second quantum dot are distinguishable; and

quantitating the target nucleic acid by analyzing the detected optical characteristic, thereby detecting said single copy of said target nucleic acid.

18. (Once amended) A method of detecting the presence of a single copy of a target nucleic acid in a sample, said method comprising:

transcribing said single copy of said target nucleic acid using a primer comprising an immobilizable label to form an immobilizable target nucleic acid;

PATENT

immobilizing said immobilizable target nucleic acid on a solid support to form an immobilized target nucleic acid;

A5

contacting said immobilized target nucleic acid with a sequence-tagged hybridization probe comprising a sequence complementary to a portion of said target nucleic acid;

detecting an optical characteristic of a quantum dot conjugate comprising a first quantum dot, a second quantum dot, and a nucleic acid sequence complementary to a portion of said sequence-tagged hybridization probe, wherein said first quantum dot and said second quantum dot are distinguishable, thereby detecting said single copy of said target nucleic acid.

A6

19. (New) The method as in claim 1, wherein said optical characteristic is detected by coincidence detection.

20. (New) The method as in claim 1, further comprising resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single copy of said target nucleic acid from an optical characteristic of a quantum dot not attached to said single copy of said target nucleic acid

21. (New) The method as in claim 17, further comprising resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single copy of said target nucleic acid from an optical characteristic of a quantum dot not attached to said single copy of said target nucleic acid.

22. (New) The method as in claim 18, further comprising resolving said optical characteristic of said quantum dot conjugate from an optical characteristic of a quantum dot conjugate not attached to said immobilized target nucleic acid

23. (New) The method as in claim 1, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence

emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

A4

24. (New) The method as in claim 1, wherein said optical characteristic is fluorescence.

25. (New) The method as in claim 1, wherein said first quantum dot and said second quantum dot are visually distinguishable as a first color and a second color, respectively.

26. (New) The method as in claim 25, wherein said first color and said second color combine to form a third color that is visually or electronically distinguishable from both said first color and said second color.

27. (New) A method of selecting a mutant DNA away from a wild type DNA, said method comprising:

contacting mutant DNA attached to a first and a second sequence-tagged hybridization probe with a first and a second oligonucleotide tag comprising a sequence complementary to said first and second sequence-tagged hybridization probes and conjugated to a first quantum dot and a second quantum dot, wherein said first quantum dot and said second quantum dot are distinguishable;

contacting wild type DNA attached to a third and a fourth sequence-tagged hybridization probe with a third and a fourth oligonucleotide tag comprising a sequence complementary to said third and fourth sequence-tagged hybridization probes and conjugated to a third quantum and a fourth quantum dot, wherein said third quantum dot and said fourth quantum dot are distinguishable; and

detecting an optical characteristic of the quantum dots, whereby detection of said optical characteristic of said first quantum dot and said second quantum dot

PATENT

detects the mutant DNA and detection of said optical characteristic of said third quantum dot and said fourth quantum dot detects wild type DNA.

A6

28. (New) The method as in claim 27, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof, and

wherein said third quantum dot and said fourth quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

29. (New) The method as in claim 27, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is fluorescence; and

wherein said third quantum dot and said fourth quantum dot are distinguishable by an optical characteristic which is fluorescence.

30. (New) The method according to claim 27, wherein said first quantum dot, said second quantum dot, said third quantum dot, and said fourth quantum dot are visually or electronically distinguishable as a first color, a second color, a third color, and a fourth color respectively.

31. (New) The method according to claim 30, wherein said first color and said second color combine to form a visually or electronically distinguishable color different from both said first color and said second color, and